

Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

Clinical profile and molecular detection of *Chlamydia trachomatis* in follicular conjunctivitis: Insights from Egypt

Kholoud M. Abd El-Moneem¹, Mohamed N. Hamza², Shereen B. El-Sayed¹, Amal M. Soliman^{1*}

1- Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

2- Ophthalmology Department, Faculty of Medicine, Ain-Shams University, Cairo, Egypt.

ARTICLE INFO

Article history:

Received 9 May 2024

Received in revised form 5 June 2024

Accepted 10 June 2024

Keywords:

Chlamydia trachomatis

Follicular conjunctivitis

Molecular detection

16S rRNA

Egypt

ABSTRACT

Background: *Chlamydia trachomatis* infection is a significant cause of follicular conjunctivitis worldwide, particularly in regions where trachoma is endemic. **Aim of the work:** This study investigates the prevalence, clinical significance, and molecular diagnosis of *Chlamydia trachomatis* in patients with follicular conjunctivitis, shedding light on important demographic and clinical associations identifying knowledge gaps and proposing avenues for future research and public health interventions. **Methodology:** Forty patients presenting with follicular conjunctivitis at Ain Shams University hospitals, Egypt. Conjunctival scraping samples were collected, and DNA extraction was performed for amplification targeting the 16S rRNA gene of *Chlamydia trachomatis* by conventional PCR. **Results:** The key finding is that 75% of conjunctival samples tested positive for the presence of the *C. trachomatis* 16S rRNA gene. Significant associations were found between PCR positivity and demographic characteristics such as gender ($P < 0.005$) with an equal distribution of 20 males and 20 females and age ($P = 0.021$) ranged from 18 to 64 years, with a median age of 26 years. Clinical symptoms were also strongly associated with positive PCR results ($P < 0.005$) including redness, foreign body sensation, irritation, itching, tearing, burning sensation, and blurred vision, along with unilateral and/or bilateral site of infection. **Conclusion:** These results underscore the clinical significance of *C. trachomatis* in follicular conjunctivitis and stress the necessity for targeted interventions and public health initiatives. Future efforts should focus on the development of a rapid, simple, and cost-effective diagnostic test to curtail the spread of *C. trachomatis* infection and targeting its proper management.

Introduction

C. trachomatis is a bacterial pathogen that is known to cause a variety of illnesses in humans worldwide, such as respiratory, urogenital, and ocular infections [1].

Follicular conjunctivitis linked to trachoma is one of the ocular manifestations of the disease that is a leading cause of preventable blindness globally, especially in areas with limited access to healthcare settings [2,3].

DOI: 10.21608/MID.2024.288481.1937

* Corresponding author: Amal M. Soliman

E-mail address: amal.soliman@med.asu.edu.eg

© 2020 The author (s). Published by Zagazig University. This is an open access article under the CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

Research gaps include the need for comprehensive epidemiological studies in different geographical regions estimating the extent of affection by this type of infection especially in endemic areas like Egypt. In turn, the prevalence rate can be reflected to prevention and control campaigns to avoid the complications and antibiotic resistance. Certain serovars of *C. trachomatis* produce trachoma, which is characterized by persistent conjunctival inflammation that, if left untreated, can lead to scarring and visual impairment. Although the presence of follicles is frequently used to make a clinical diagnosis [4,5].

Trachoma is a significant cause of blindness globally which is caused by *Chlamydia trachomatis* serovars A, B, Ba and C that mainly affect the eyes. Also, these serovars are rarely isolated from Chlamydial genital tract infections. Based on the antigenic diversity of the OMP A genes, which encode the bacterium's main outer membrane protein (MOMP), *C. trachomatis* is divided into 15 different serovars which exhibit distinct and well-documented tissue tropisms [6].

Unfortunately, Africa is the worst affected continent: 18 million cases of active trachoma (85% of all cases globally) are thought to exist in 29 of the 47 countries in World Health Organization's (WHO) African Region. Ethiopia and South Sudan have the highest prevalence of active trachoma. Beyond the disability, distress, isolation, and stigma that it causes, the economic burden of trachoma on affected individuals and communities is enormous, costing between US\$ 2.9 - 5 billion annually [7].

In 2003, trachoma rapid assessments (TRAs) were undertaken in 15 villages of El-Fayoum Governorate. The village-level proportions of 2–10 years old children observed to have active trachoma ranged from 16% to 85% [8].

Active trachoma is determined by the follicles existing and inflammation in the conjunctiva of the upper tarsal. An immunopathological inflammatory response resulting in tissue fibrosis is thought to be triggered by recurrent or persistent infection. This can leave the upper tarsal conjunctiva severely scarred and cause the eyelashes to turn inward, which cause scratch the eyeball and corneal opacity then blindness [5,9].

An estimated ninety-two million new sexually transmitted infections (STI) with *C. trachomatis* were reported annually [10]. Based on

the 2018 global STI surveillance from the WHO, the global estimation of new *C. trachomatis* cases in 2016 was 127 million [11].

Although serovars D-K are not linked to blinding trachoma, they are a major universal cause of urogenital tract infections. On the other hand, serovars D-K can infect the eyes of adults and through the accidental contact with vaginal discharge or if newborns contract the pathogen while passing through an infected birth canal [12].

The infection spreads mostly by three routes: direct contact with the eye and nasal secretions of an infected individual, contact with fomites, and eye-seeking flies contaminated by ocular or nasal secretions harboring the bacteria *C. trachomatis*, specifically *Musca sorbens* [13].

Mostly follicular conjunctivitis is diagnosed from a clinical perspective, and empirically administered antibiotics are subsequently used as treatment, which contributes to the emergence of antibiotic resistance. When observed in individuals who reside in trachoma-endemic regions, the follicles are predictive of the disease but are not pathognomonic. There are additional causes of follicular conjunctivitis, including bacterial infections (especially those caused by *Moraxella* spp. and *Streptococcus pneumoniae*), viral infections (of which the adenovirus is the most prevalent), and toxic conjunctivitis secondary to topical medications. Therefore, it is possible that this clinical sign is not specific to Chlamydial infection [14,15].

There are two principal forms of *Chlamydia* species in the developmental cycle that have been identified in tissue culture systems [16]. The extracellular, infectious, metabolically dormant form known as the "elementary body" is characterized by condensed genomic DNA and limited or absent transcription. The elementary body changes into the reticulate body form upon infection of the host cell, becoming metabolically active and dividing by binary fission. Given that chlamydial 16S rRNA is among the genes that are prominently expressed during the transition from elementary bodies to reticulate bodies that actively replicate, high-level expression of this gene may serve as a marker for organisms that are viable or actively replicating [17,18].

16S rRNA gene is vital to the organism's normal function as a part of the chlamydial ribosome. In addition to serving as a marker of

microbial metabolism in vitro, its expression corresponds with the load of chlamydial organisms in the sample [18].

The prognosis is favourable for high-risk groups when antibiotic therapy and education are combined [19]. So, sensitive, specific, rapid techniques for screening and early diagnosis of chlamydial infections have been developed due to the rising prevalence of chlamydial infection [20]. Also, for prevention of serious complications, early diagnosis is essential particularly when appropriate treatment is available [21].

Bacterial isolation in tissue culture media, which is time-consuming and slow, is usually the basis for diagnosis. So, its detection was widely replaced by polymerase chain reaction (PCR) [20].

In detecting *C. trachomatis* infection, molecular techniques provide enhanced sensitivity and specificity. Tests with high analytical sensitivity are necessary for its directly detection in medical samples due to the intracellular lifestyle of the bacteria and its ability to induce low-grade replication infections that can lead to persistent infections. The most sensitive assays for its detection are nucleic acid amplification tests (NAATs), which have a specificity comparable to that of cell culture and are hence the method of choice [22].

Therefore, this study aims to molecularly detect the presence of *C. trachomatis* 16S rRNA gene in conjunctival samples which was chosen for its specificity and its conserved nature across strains, enabling accurate detection of the pathogen and investigate its association with clinical features and demographic parameters in patients complaining of follicular conjunctivitis in an endemic region like Egypt.

Methods

Ethical approval

Ethical approval (FMASU MS 136/2023) was obtained according to the Ethical Committee of Scientific Research, Faculty of Medicine, Ain Shams University. Written consent was obtained from the participants involved in the study.

Subjects

The sample size was determined based on statistical considerations to achieve adequate power for detecting significant associations and fulfilling study objectives. Sample size was performed in the community, environmental and occupational

medicine department, faculty of medicine, Ain Shams University.

This study was a cross-sectional study conducted at the outpatient ophthalmology clinic at Ain-Shams University Hospitals, Egypt, from March 2023 to November 2023. Patients were selected based on clinical presentation with follicular conjunctivitis using established criteria such as characteristic symptoms and signs upon examination. It was performed on forty clinical specimens collected from patients complain from one or more symptoms of the following: pink or red eye(s), increased tear production, itching, irritation, burning or foreign body sensation in the eye(s). They were examined for follicles on the upper &/or lower tarsal & forniceal conjunctiva as a clinical sign. We excluded patients clinically diagnosed with cicatricial stages of trachoma, other causes of conjunctivitis such as mucopurulent conjunctivitis, allergic conjunctivitis, also patients with any sign suggestive of viral causes like dendritic corneal ulcer of herpes simplex virus, etc.

Clinical diagnosis

The clinical examination for the signs of follicular conjunctivitis and the diagnosis was performed by an ophthalmic consultant.

Sample Collection

Conjunctival scraping samples collected by an ophthalmic consultant from adults clinically diagnosed with follicular conjunctivitis for molecular analysis in the medical microbiology laboratory at faculty of medicine, Ain Shams University. The samples were collected before the administration of empirical antibiotic therapy from the upper tarsal conjunctival surfaces after applying topical anaesthesia. Then preserved on Dacron swab in Universal Transport Medium (UTM) and stored until their processing.

DNA extraction

DNA was extracted and amplified from all samples using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.

DNA amplification with 16S rRNA gene primers

Primers were designed with the aid of the Vector NTI® software (Thermo Fisher Scientific, Waltham, MA, USA) and primer-BLAST (<https://www.ncbi.nlm.nih.gov/tools/primerblast>).

The primers sequence used in this study was (F/ 5'- AGTGGCGGAAGGGTTAGTAATG -

3') (R/ 5'- TCACATAGACTCTCCCTTAACCGA-3'), with amplicon size of 156 bp [23].

The chosen primers targeted the gene of *C. trachomatis* 16s rRNA due to their unique pattern of conservative and hypervariable motives regions of the published DNA sequences [24].

The reaction volume was 25 μ L containing 12.5 μ L ready-made TAQ COSMO PCR RED Master Mix (Willowfort, UK), 1 μ L of primers (0.5 μ mol/L of each primer), 5 μ L of DNA template and 6.5 μ L complete with distilled H₂O.

DNA amplification was performed under the following thermal conditions: pre-denaturation, 95 °C for 10 min; amplification for 40–45 cycles of 95 °C for 10 sec, 62 °C for 20 sec, 72 °C for 30 sec [23], by using ready-made master mix Red TAQ COSMO PCR RED Master Mix (Willowfort, UK). To avoid the potential of contamination, the sample preparation and PCR amplification product were performed in separate rooms.

PCR analysis

All PCR products were subjected to electrophoresis on agarose gel along with a 100 bp DNA marker. An amount of 5 μ L of each PCR mixture was separated in 1.5% agarose gel, containing 3 μ l ethidium bromide, and viewed under the gel documentation system.

A result was considered positive when a band of the size 156 bp was visible in the gel corresponding to the positive control.

Standard procedures for reducing contamination were strictly followed. Each assay included positive and negative controls; negative control contained sterile distilled water.

Statistical analysis

Data were collected, revised, coded, and entered the Statistical Package for Social Science (IBM SPSS) version 27. The comparison between groups regarding qualitative data was done by using the Chi-square test (*) and/or Fisher exact test when the expected count in any cell was found to be less than 5.

The comparison between two independent groups with quantitative data and non-parametric distribution was done by using the Mann-Whitney test (\neq).

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

P-value > 0.05: Non-significant (NS)

P-value < 0.05: Significant (S)

P-value < 0.01: Highly significant (HS).

Results

A total of forty conjunctival samples were obtained from patients clinically diagnosed with follicular conjunctivitis, with an equal distribution of twenty males and twenty females. The range of ages was 18 to 64 years, with a median age of 26 years as shown in (Table 1).

PCR results revealed thirty samples (75%) tested positive for *C. Trachomatis*, while ten samples (25%) tested negative as shown in Figure (1B). Significant associations were found between PCR positivity and demographic factors such as gender and age, as well as clinical manifestations of follicular conjunctivitis. The detailed results were presented in the following tables and figures.

Table 1 shows that most of the patients (82.5%) were aged 18-39 years old with equal distribution between 20 males and 20 females. The median age was 26.

Figures (1A & 1B) illustrate gender distribution and the conventional PCR results among the collected conjunctival samples.

Table 2A highlights a highly significant association ($P < 0.005$) between PCR results and gender. Females exhibited a higher positivity rate (66.7%) compared to males (33.3%).

Table 2B presents the relationship between PCR results and age. A significant association was found ($P = 0.021$), indicating a higher positivity rate in young adults (76.7%).

The detailed results of conventional PCR compared to the age of those patients revealed a significant relationship ($P < 0.05$) between PCR results and the median age

Table 3 illustrates the results of conventional PCR compared to symptoms of the patients. There was a highly statistical significance ($P < 0.005$) between the results of the conventional PCR assay and the patients' symptoms.

Table 4 presents the relationship between PCR results and the site of infection. A highly significant association was found ($P < 0.005$), indicating a higher positivity rate in bilateral eye infection (96.7%).

Agarose gel electrophoresis results as shown in Figures (2A & 2B) confirmed the presence of *C. trachomatis* 16S rDNA in positive samples.

Figure 3 shows the relationship between results of conventional PCR in the patients in the study, and duration of symptoms, there was non-significant relationship between days of complaints and the positive results of PCR.

Table 1. Demographic data of the patients included in the study.

		Total No. = 40	Percentage (%)
Gender	Female	20	50
	Male	20	50
Age	Median (IQR)	26 (18 – 36.5)	
	Range	18 – 64	
Age Group	Young Adults (18-39)	33	82.5
	Middle-aged adults (40-59)	4	10
	Old adults (60-64)	3	7.5

Table 2A. Gender distribution among the patients in the study with positive and negative PCR results.

Gender	PCR results	No.	Percentage (%)	Test value	P-value	Significance
Female	Positive	20	66.7	13.333*	<0.005	HS
	Negative	0	0.0			
Male	Positive	10	33.3			
	Negative	10	100			

Table 2B. Age distribution among the patients included in the study with positive and negative PCR results.

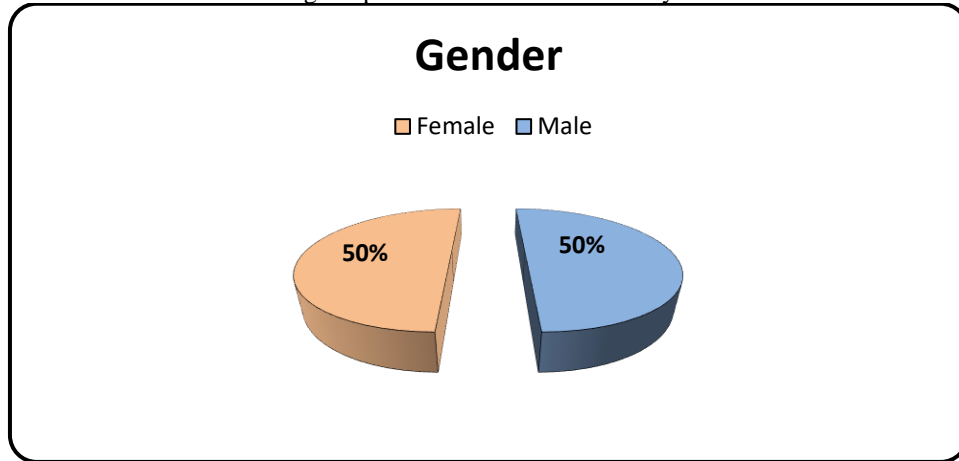
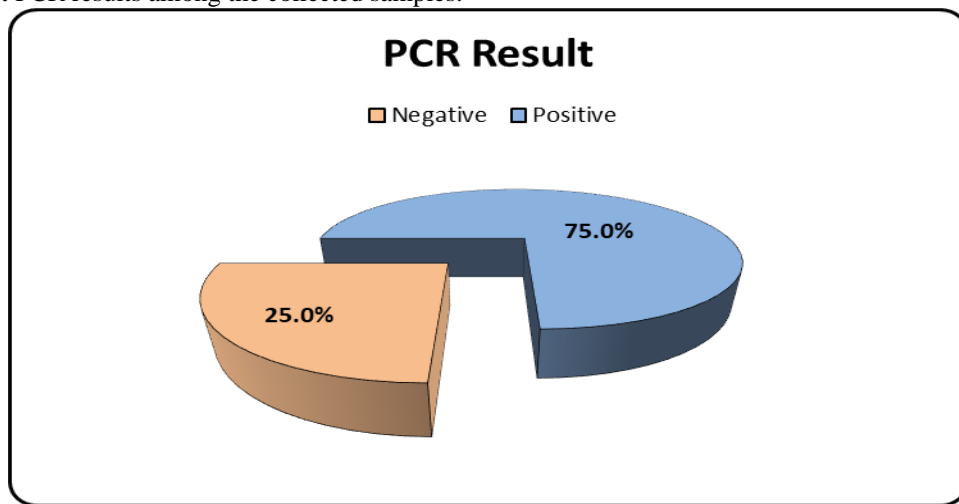
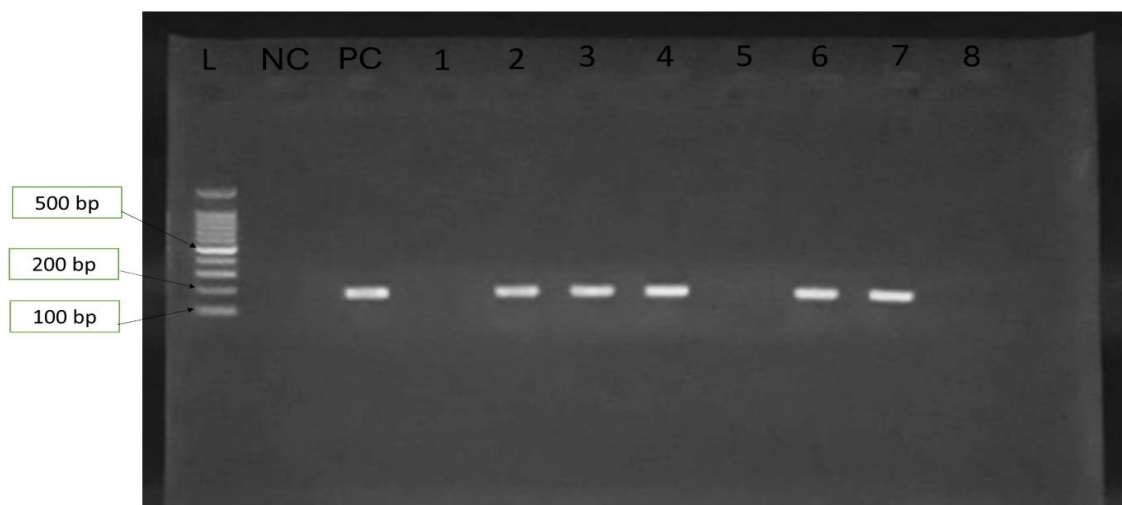
Age Group	PCR results	No.	Percentage (%)	Test value	P-value	Significance	
Young Adults (18-39)	Positive	23	76.7	2.828*	0.243	NS	
	Negative	10	100				
Middle-aged Adults (40-59)	Positive	4	13.3				
	Negative	0	0.0				
Old Adults (60-64)	Positive	3	10				
	Negative	0	0.0				
Age	Median (IQR)	Positive	33.5 (19 – 39)		-2.314≠	0.021	S
		Negative	18.5 (18 – 29)				
	Range	Positive	18 – 64				
		Negative	18 – 35				

Table 3. Relation between the symptoms among the patients included in the study & the results of conventional PCR assay.

Symptoms		Total No.	PCR Results				Test value	P-value	Significance
			Negative		Positive				
			No.	%	No.	%			
Redness	Yes	39	0	0.0	30	100	40	<0.005	HS
			9	90	0	0.0			
	No	1	1	10	0	0.0			
			0	0.0	0	0.0			
Foreign body sensation	Yes	37	0	0.0	29	96.7	40	<0.005	HS
			8	80	0	0.0			
	No	3	2	20	0	0.0			
			0	0.0	1	3.3			
Irritation	Yes	37	0	0.0	29	96.7	40	<0.005	HS
			8	80	0	0.0			
	No	3	2	20	0	0.0			
			0	0.0	1	3.3			
Itching	Yes	36	0	0.0	28	93.3	40	<0.005	HS
			8	80	0	0.0			
	No	4	2	20	0	0.0			
			0	0.0	2	6.7			
Tearing	Yes	35	0	0.0	30	100	40	<0.005	HS
			5	50	0	0.0			
	No	5	5	50	0	0.0			
			0	0.0	0	0.0			
Burning sensation	Yes	15	0	0.0	10	33.3	40	<0.005	HS
			5	50	0	0.0			
	No	25	5	50	0	0.0			
			0	0.0	20	66.7			
Blurred Vision	Yes	10	0	0.0	4	13.3	40	<0.005	HS
			6	60	0	0.0			
	No	30	4	40	0	0.0			
			0	0.0	26	86.7			

Table 4. Relation between site of infection in one eye or both among the patients in the study with PCR results.

Site of infection	PCR results	No.	Percentage (%)	Test value	P-value	Significance
Unilateral	Total	8	20	20.833*	<0.005	HS
	Positive	1	3.3			
	Negative	7	70			
Bilateral	Total	32	80			
	Positive	29	96.7			
	Negative	3	30			

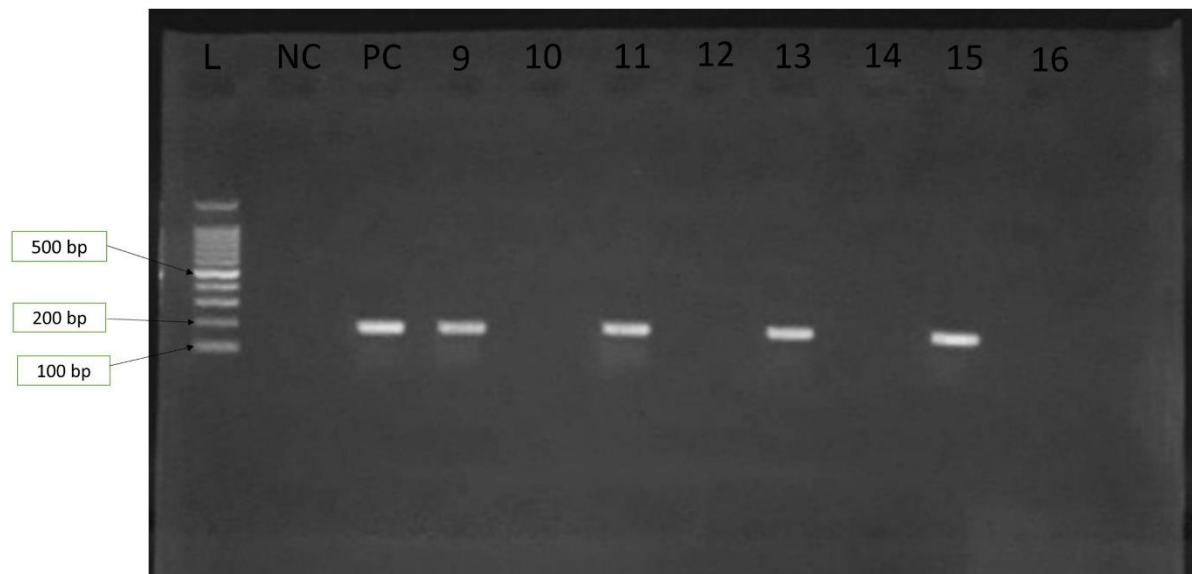
Figure 1A. Gender distribution among the patients included in the study.**Figure 1B.** PCR results among the collected samples.**Figure 2A.** Agarose gel electrophoresis of the amplified products of the first run PCR indicating the existence of *C. trachomatis* 16S rDNA (156 bp).

Lanes 2, 3, 4, 6, 7: correspond to the positive bacterial DNA yield.

Lane PC: corresponds to positive control.

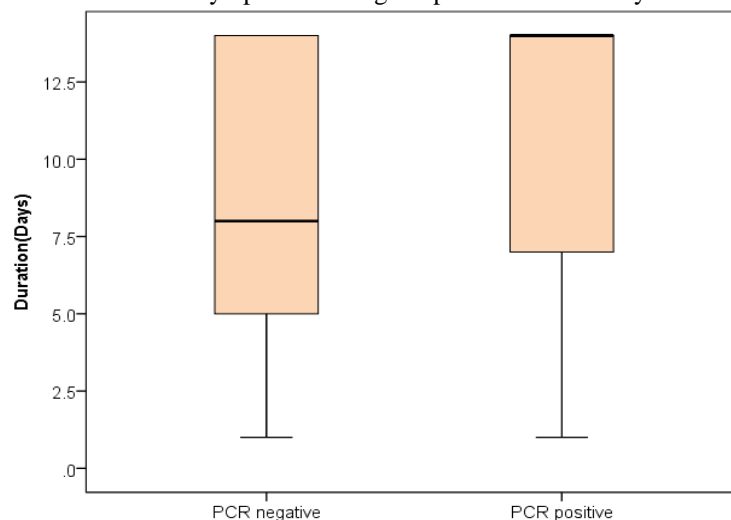
Lane NC: corresponds to negative control (distilled water).

Figure 2B. Agarose gel electrophoresis of the amplified products of the second run PCR indicating the existence of *C. trachomatis* 16S rDNA (156 bp).



Lanes 9, 11, 13, 15: correspond to the positive bacterial DNA yield.
Lane PC: corresponds to positive control.
Lane NC: corresponds to negative control (distilled water).

Figure 3. Relation between duration of symptoms among the patients in the study and PCR results.



Discussion

C. trachomatis is one of the most preventable causes which lead to chronic conjunctivitis and blindness. Annually about one million cases reported that could be infected by *C. trachomatis* according to the Centre for Disease Control and Prevention (CDC). At the beginning of the acute phase of the conjunctival infection, it is important to administer the specific antibiotic therapy, but it is also important to choose a correct method to detect the presence of *C. trachomatis*, such as NAATs recommended by CDC [25,26].

The present study findings underscore the high prevalence of *C. trachomatis* (75%) in follicular conjunctivitis among clinically diagnosed adult patients and highlights the importance of early molecular detection of 16S rRNA gene by conventional PCR.

Our findings align with existing literature on the prevalence of *C. trachomatis* in follicular conjunctivitis confirming the global relevance as a causative agent in follicular conjunctivitis [27,28]. The high prevalence reported in our study is consistent with trachoma being a major public

health problem, resonating with data from various regions [18,29,30].

Our findings coincide with the results of the study performed in Giza, Egypt by **Abdelfattah et al.** [31] who detected *C. trachomatis* in 65.5% and 76% of dry eye disease (DED) patients by direct fluorescent antibody (DFA) and PCR methods, respectively. The study was conducted on 58 patients of age range 20-50 years diagnosed with DED confirmed by Schirmer I test and tear breakup time. Also, our findings align with the results documented by **Fan et al.** [27] who found that the PCR results were positive in 31 out of 49 specimens of patients with conjunctivitis by 63.3%.

In addition, **Gallenga et al.** [32] detected *C. trachomatis* in 82 patients out of 89 by 94.25% with several clinical manifestations of different degrees of conjunctivitis by PCR.

Another study was done in Giza, Egypt by **Khattab and Abdelfattah** [28] found that ocular *C. trachomatis* was present in 60% of symptomatic women by PCR from 100 samples.

While another study was done by different techniques in Mansoura, Egypt by **Abd El Samea et al.** [33] who evaluated the direct antigen detection of *C. trachomatis* in conjunctival scrapings by enzyme linked immunosorbent assay (ELISA) revealed that there was insignificant difference between active and cicatricial ($P>0.05$). Also, evaluated *C. trachomatis* antibodies in the sera of patients, revealed insignificant higher titre of *C. trachomatis* antibodies in the sera of active than cicatricial cases. However, direct antigen detection test and serodiagnosis of *C. trachomatis* IgM by ELISA are more reliable than ELISA IgG in diagnosis of active trachoma infection. ELISA IgG is a reliable method in the serodiagnosis of cicatricial phase of trachoma.

Whereas a study was done by another approach in Mansoura, Egypt by **Abd El-Aal et al.** [34] on 40 patients clinically diagnosed with chlamydial conjunctivitis. *C. trachomatis* was detected by direct Giemsa stain in 1/40 (2.5%) of samples but isolated in the Vero cell line and identified in 4/40 samples (10%) by Giemsa and iodine stains or Gen-Probe. However, ELISA identified only 2/40 (5%) of total samples after culture. Direct Giemsa stain provided low sensitivity (25%) and a high specificity (100%) compared with cell cultures, with high statistically significant differences between both tests (P -value <0.001). *C. trachomatis* was detected in a

relatively high rate of acute follicular conjunctivitis patients as detected with culture on the Vero cell line. Identification of *C. trachomatis* in culture was 100% by Giemsa and iodine stains or Gen-Probe but only 50% by ELISA.

The discrepancy between the results obtained by the different techniques for detection of *C. trachomatis* is consistent with the value of using molecular techniques that offer higher sensitivity and specificity rates than cell culture, DFT and ELISA that facilitate accurate diagnosis.

A significant association between age and *C. trachomatis* prevalence ($P < 0.05$) with a higher positivity rate in young adults aged between 18-39 years old was found in the present study that provides insights into the age-specific vulnerability to this infection. This finding supports the existing literature stated by **Petrovay et al.** [35] that also supports the higher positive rates in females more than males and emphasizes the importance of considering gender and age as contributing factors in follicular conjunctivitis aetiology especially in adult age group. However, this finding goes against the results obtained by **Hamed** [36] who found no significant relationship between PCR results and age.

In addition, our study revealed a significant gender-based difference, with females exhibiting a higher prevalence of *C. trachomatis* compared to males (66.7% versus 33.3% respectively) ($P < 0.01$). This contrasts with **Sharma et al.** [37] who stated that males had significantly higher positivity rate than females in the age group of 31-60 years ($P < 0.05$) using *C. trachomatis* antigen detection suggesting the climatic changes have a role in this finding.

A previous cross-sectional study agreed with our findings in assessing patients presenting with acute conjunctivitis in Giza, Egypt was done by **Mowafy et al.** [38] who revealed that the prevalence of trachoma among females was higher than males by 80% and 63% for Immunoglobulin M and Immunoglobulin G by ELISA respectively with a significant relationship between Immunoglobulin G and female sex.

This was in accordance with **Courtright & West** [39] who also found a high prevalence of active trachoma in females, then those females acquire severe conjunctival scarring by the age of 40.

In addition, **Melese et al.** [40] collected survey data from trachoma-endemic settings showed that trachoma-related blindness is two to four times higher in women compared to men.

As well, our study established a significant correlation between symptoms and PCR results, reinforcing the clinical relevance of *C. trachomatis* in follicular conjunctivitis. Symptoms such as redness founds in 39 (97.5%), foreign body sensation in 37 (92.5%), irritation in 37 (92.5%), itching in 36 (90%), tearing in 35 (87.5%), burning sensation in 15 (95%), and blurred vision in 10 (25%), were strongly associated with positive PCR results ($P < 0.01$), guiding clinicians in early diagnosis and targeted treatment.

This was close to results obtained by **Hamed** [36] who found that redness occurs in 99%, burning in eyes 95%, eye discharge 65%, lacrimation 86% and foreign body sensation 61%.

In contrast with a previously mentioned study in Giza, Egypt done by **Mowafy et al.** [38] who revealed that the main presenting symptom among 302 patients was lacrimation 26.8% followed by discharge 25.8% respectively. Pain and redness were equally distributed 21.5% and 21.9% respectively. The least presenting complaint was itching 1% among the studied sample. Half of the studied group had associated symptoms with the main complaint 50.3% in the form of redness, lacrimation, and foreign body sensation, respectively. On the other hand, they found that 78.1% of patients presented with bilateral eye affection in comparison to 21.9% who had unilateral affection, which coincides with our findings (80% bilateral versus 20% unilateral).

Our study elucidates a highly significant correlation between the site of infection and PCR results ($P < 0.01$). This finding indicates that the active stage causes bilateral eye affection which coincides with the results of the previously mentioned study done in Mansoura, Egypt by **Abd El Samea et al.** [33] who found that all antigen positive cases in group I (included twenty active cases) were bilaterally affected. The prevalence of bilateral eye infection suggests a potential role of *C. trachomatis* in acute, recurrent, and chronic follicular conjunctivitis episodes, emphasizing the need for early diagnosis.

The high prevalence of *C. trachomatis* infection detected in this study highlights its clinical significance in follicular conjunctivitis in adult age.

The observed associations with demographic parameters and symptoms provide valuable insights for clinical management and public health interventions. So, molecular detection offers enhanced sensitivity and specificity, facilitating early accurate diagnosis.

Implications for Public Health

Our findings have implications for public health, emphasizing the importance of early diagnosis to control the spread of *C. trachomatis* ocular infections and lessen the burden of its sequelae especially cicatricial complication and finally blindness.

Limitations and future directions

Limitations include the single-centre area, and reliance on PCR for diagnosis, which may limit generalizability to other populations and settings.

This study results align with existing literature on the prevalence of *C. trachomatis* in follicular conjunctivitis and underscore the need for future larger, multicentre studies to validate findings and apply novel interventions.

Potential mechanisms may include variations in exposure risk, immune response, and pathogen virulence, necessitating further research to elucidate underlying factors and higher sample size to be investigated to estimate the prevalence in other geographical areas.

Conclusion

The study highlights the high prevalence of *C. trachomatis* in follicular conjunctivitis and its associations with demographic and clinical factors, emphasizing the importance of targeted interventions.

The findings contribute to the broader understanding of ocular infections caused by *C. trachomatis* and its role in follicular conjunctivitis and the need for recruitment of public health interventions to prevent the spread of infection and the subsequent blindness as a terminal complication of trachoma.

Recommendations

Further research is warranted to explore the factors contributing to the observed gender and age disparities in *C. trachomatis* prevalence. Additionally, routine molecular screening for ocular pathogens, including *C. trachomatis*, may improve our understanding of ocular microbiology and aid in early diagnosis and proper management. Public health efforts should focus on raising awareness,

implementing preventive strategies, and ensuring access to effective treatment to reduce the burden of *C. trachomatis*-related ocular diseases.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained according to the Ethical Committee of Scientific Research, Faculty of Medicine, Ain Shams University (FMASU MS 136/2023).

Consent to participate written consent was obtained.

Availability of data and materials

The data presented in this study are available upon request from the corresponding author.

Conflicts of interest: None.

Financial disclosure: None

Funding: This research received no specific grant from a governmental, private, or non-profit organization.

Author Contributions

A.M. Soliman, S. B. El-Sayed, M. N. Hamza, and K. M. Abd El-Moneem contributed to the idea and the design of the study.

A.M. Soliman, K. M. Abd El-Moneem and M. N. Hamza performed the practical work.

A.M. Soliman, S. B. El-Sayed and M. N. Hamza analyzed and interpreted the data.

A.M. Soliman and K. M. Abd El-Moneem wrote the original draft.

A.M. Soliman, S. B. El-Sayed, M. N. Hamza, and K. M. Abd El-Moneem reviewed and edited the manuscript.

All authors read and approved the final manuscript.

References

- 1- Satpathy G, Behera H, Ahmed N. Chlamydial eye infections: Current perspectives. *Indian J Ophthalmol* 2017; 65(2):97. Doi: 10.4103/ijo.IJO_870_16
- 2- Baneke A. Review: Targeting trachoma: Strategies to reduce the leading infectious cause of blindness. *Travel Med Infect Dis* 2012;10(2):92–6. Doi: 10.1016/j.tmaid.2012.01.005
- 3- Getachew Atsbha S. A review of the prevalence of Trachoma, its Control program, and challenges in Ethiopia. *International Journal of Drug Regulatory Affairs* 2023; 11(1):54–60. Doi: 10.22270/ijdra.v11i1.585
- 4- Taylor HR, Burton MJ, Haddad D, West S, Wright H. Trachoma. *The Lancet* 2014; 384(9960):2142–52. Doi: 10.1016/S0140-6736(13)62182-0
- 5- Solomon AW, Burton MJ, Gower EW, Harding-Esch EM, Oldenburg CE, Taylor HR, et al. Trachoma. *Nat Rev Dis Primers* 2022; 8(1):32. Doi: 10.1038/s41572-022-00359-5.
- 6- Schachter J, West SK, Mabey D, Dawson CR, Bobo L, Bailey R, et al. Azithromycin in control of trachoma. *The Lancet* 1999;354(9179):630–5. Doi: 10.1016/S0140-6736(98)12387-5
- 7- World Health Organization. WHO Alliance for the Global Elimination of Blinding Trachoma by the year 2020: Progress report on elimination of trachoma, 2013. *Weekly Epidemiological Record*. WHO 2014; 89(39):421-8. ISSN 0049-8114.
- 8- Ezz al Arab G. Trachoma rapid assessment and planning for intervention: a pilot study in Fayoum Governorate. *Partnership in Development Research American University in Cairo. Research Brief* 2003; 9.
- 9- Hu VH, Holland MJ, Burton MJ. Trachoma: Protective and Pathogenic Ocular Immune Responses to *Chlamydia trachomatis*. *PLoS Negl Trop Dis* 2013;7(2): e2020. Doi: 10.1371/journal.pntd.0002020
- 10- Qayum M, & Khalid-bin-Saleem M. Prevalence of *Chlamydia trachomatis* among asymptomatic women. *Journal of Ayub Medical College Abbottabad*. 2013;1;25(1-2):28-30. Doi: www.lexic.us/definition-. PMID:25098047.

- <http://www.ayubmed.edu.pk/JAMC/25-1/Mamuna.pdf>.
- 11-Huai P, Li F, Chu T, Liu D, Liu J, Zhang F. Prevalence of genital *Chlamydia trachomatis* infection in the general population: a meta-analysis. *BMC Infect Dis* 2020; 20:589. Doi: 10.1186/s12879-020-05307-w
 - 12-Lesiak-Markowicz I, Schötta A-M, Stockinger H, Stanek G, Markowicz M. *Chlamydia trachomatis* serovars in urogenital and ocular samples collected 2014–2017 from Austrian patients. *Sci Rep* 2019;9(1):18327. Doi: 10.1038/s41598-019-54886-5
 - 13-Ahmad B, & Patel BC. Trachoma. *StatPearls* 2022. [QxMD MEDLINE Link].
 - 14-THYGESON P. Aetiology and differential diagnosis of non-trachomatous follicular conjunctivitis. *Bull World Health Organ* 1975;16(5):995–1011. PMID: 13472441; PMCID: PMC2538254.
 - 15-Salmon JF. *Clinical Ophthalmology a Systematic Approach* [Internet] 2020. doi: www.elsevier.com/permissions. Doi: 10.1016/B978-0-7020-7711-1.00023-6
 - 16-Barron AL. *Microbiology of Chlamydia*. CRC Press 2019. Doi: 10.1201/9780429276521
 - 17-Engel JN, & Ganem D. Chlamydial rRNA operons: gene organization and identification of putative tandem promoters. *J Bacteriol* 1987;169(12):5678–85. Doi: 10.1128/jb.169.12.5678-5685.1987
 - 18-Mathews SA, Volp KM, Timms P. Development of a quantitative gene expression assay for *Chlamydia trachomatis* identified temporal expression of σ factors. *FEBS Lett* 1999; 458(3):354–8. Doi: 10.1016/S0014-5793(99)01182-5
 - 19-Grygiel-Górniak B, & Folga BA. *Chlamydia trachomatis*—An Emerging Old Entity? *Microorganisms* 2023;11(5):1283. Doi: 10.3390/microorganisms11051283
 - 20-Fallah F, Kazemi B, Goudarzi H, Badami N, Doostdar F, Ehteda A, et al. Detection of *Chlamydia trachomatis* from Urine Specimens by PCR in Women with Cervicitis 2005;34(2):20-26. Available from: <https://sid.ir/paper/272070/en>
 - 21-Boyadzhyan B, Yashina T, Yatabe JH, Patnaik M, Hill CS. Comparison of the APTIMA CT and GC Assays with the APTIMA Combo 2 Assay, the Abbott LCx Assay, and Direct Fluorescent-Antibody and Culture Assays for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J Clin Microbiol* 2004;42(7):3089–93. Doi: 10.1128/JCM.42.7.3089-3093.2004
 - 22-Shetty S, Kouskouti C, Schoen U, Evangelatos N, Vishwanath S, Satyamoorthy K, et al. Diagnosis of *Chlamydia trachomatis* genital infections in the era of genomic medicine. *Braz. J. Microbiol* 2021;52(3):1327–39. Doi: 10.1007/s42770-021-00533-z
 - 23-Vitrenko YA, & Deryabin OM. A dual-target strategy for the detection of *Chlamydia trachomatis* by real-time PCR. *Biopolym Cell* 2018; 34(2):117–26. Doi: 10.7124/bc.000976
 - 24-Monstein H-J, Kihlström E, Tiveljung A. Detection, and identification of bacteria using in-house broad range 16S rDNA PCR amplification and genus-specific DNA hybridization probes, located within variable regions of 16S rRNA genes. *APMIS* 1996;104(1-6):451–8. Doi: 10.1111/j.1699-0463.1996.tb00741.x
 - 25-Johnson RE, Newhall WJ, Papp JR, Knapp JS, Black CM, Gift TL, et al. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections--2002. *MMWR Recomm Rep* 2002;51(RR-15):1–38.

- 26-Solomon AW, Peeling RW, Foster A, Mabey DCW. Diagnosis and Assessment of Trachoma. *Clin Microbiol Rev* 2004;17(4):982–1011. Doi: 10.1128/CMR.17.4.982-1011.2004
- 27-Fan J, Zhang WH, Wu YY, Jing XY, Claas ECJ. Detection of infections of the eye with *Chlamydia trachomatis* by the polymerase chain reaction. *Int Ophthalmol* 1993; 17:327–30. Doi: 10.1007/BF00915738
- 28-Khattab RA & Abdelfattah MM. Study of the prevalence and association of ocular chlamydial conjunctivitis in women with genital infection by *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Candida albicans* attending outpatient clinic. *Int J Ophthalmol* 2016; 9(8):1176. Doi: 10.18240/ijo.2016.08.15
- 29-Edwards T, Smith J, Sturrock HJW, Kur LW, Sabasio A, Finn TP, et al. Prevalence of Trachoma in Unity State, South Sudan: Results from a Large-Scale Population-Based Survey and Potential Implications for Further Surveys. *PLoS Negl Trop Dis* 2012;6(4): e1585. Doi: 10.1371/journal.pntd.0001585
- 30-Ejigu M, Kariuki MM, Ilako DR, Gelaw Y. Rapid trachoma assessment in Kersa District, Southwest Ethiopia. *Ethiop J Health Sci* 2013; 23(1):1–9. eISSN: 2413-7170. print ISSN: 1029-1857.
- 31-Abdelfattah MM, Khattab RA, Mahran MH, Elborgy ES. Evaluation of patients with dry eye disease for conjunctival *Chlamydia trachomatis* and *Ureaplasma urealyticum*. *Int J Ophthalmol* 2016; 9(10):1457-1465. Doi: 10.18240/ijo.2016.10.15. PMID: 27803864; PMCID: PMC5075662.
- 32-Gallenga PE, Del Boccio M, Rapinese M, Di Iorio A, Toniato E, Martinotti S. Molecular approach by PCR is the best method to detect the presence of *Chlamydia trachomatis* and to define the true agent of ocular bacterial inflammation. *Int J Immunopathol Pharmacol* 2011; 24(2):285-96. DOI: <https://doi.org/10.1177/039463201102400202> . PMID: 21658303.
- 33-Abd El Samea ER, El Hadidy EB, El Tarshouby SM, Abd El AM. Laboratory Approach to *Chlamydia Trachomatis* Conjunctivitis. *LIFE SCIENCE JOURNAL-ACTA ZHENGZHOU UNIVERSITY OVERSEAS EDITION* 2011;8(1):329-36. ISSN:1097–8135.
- 34-Abd El-Aal AM, El Saied EM, El Sayed M, Foad MF, Elazeim DA, Mashaly M, Ahmed M. Ocular *Chlamydia trachomatis* in a tertiary hospital. *Hamdan med. J* 2015;8(2):217-24. DOI: 10.7707/hmj.386.
- 35-Petrovay F, Németh I, Balázs A, Balla E. Chlamydial conjunctivitis: prevalence and serovar distribution of *Chlamydia trachomatis* in adults. *J Med Microbiol* 2015;64(9):967–70. Doi: 10.1099/jmm.0.000115.
- 36-Hamed RA. Molecular Detection Infection with of *Trachomatis* among Patients Suffering Conjunctivitis attending AL-Saim Hospital, Wad Medani, Sudan (2018-2019) (Doctoral dissertation, University of Gezira).
- 37-Sharma A, Satpathy G, Nayak N, Tandon R, Sharma N, Titiyal JS, et al. Ocular *Chlamydia trachomatis* infections in patients attending a tertiary eye care hospital in north India: a twelve-year study. *Indian J Med Res* 2012;136(6):1004–10. PMID: 23391797; PMCID: PMC3612304.
- 38-Mowafy MA, Saad NE, El-Mofty HM, ElAnany MG, Mohamed MS. The prevalence of *chlamydia trachomatis* among patients with acute conjunctivitis in Kasr Alainy ophthalmology clinic. *Pan Afr Med J*

2014;17:151. Doi:
10.11604/pamj.2014.17.151.3818. PMID:
25374648; PMCID: PMC4219799.

39-Courtright P, & West SK. Contribution of sex-linked biology and gender roles to disparities with trachoma. *Emerg. Infect. Dis* 2004; 10(11):2012-2016. Doi:
10.3201/eid1011.040353. PMID: 15550216; PMCID: PMC3328994.

40-Melese M, Alemayehu W, Bayu S, Girma T, Hailesellasié T, Khandekar R, et al. Low vision, and blindness in adults in Gurage Zone, central Ethiopia. *Br. J. Ophthalmol* 2003; 87(6):677-80. Doi: 10.1136/bjo.87.6.677.

Abd El-Moneem KM, Hamza MN, El-Sayed SB, Soliman AM. Clinical profile and molecular detection of *Chlamydia trachomatis* in follicular conjunctivitis: Insights from Egypt. *Microbes Infect Dis* 2024; 5(3): 1176-1189.